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Tetracycline-Regulated Gene Expression Switch in *Xenopus laevis*

Patricia Ridgway, Jean-Pierre Quivy, and Geneviève Almouzni[1]

Section de Recherche UMR218 du CNRS, Institut Curie, 26 rue d'Ulm, 75231 Paris Cedex 05, France

***Xenopus* is a well-characterized model system for the investigation of biological processes at the molecular, cellular, and developmental level. The successful application of a rapid and reliable method for transgenic approaches in *Xenopus* has led to renewed interest in this system. We have explored the applicability of tetracycline-regulated gene expression, first described by Gossen and Bujard in 1992, to the *Xenopus* system. By optimizing conditions, tetracycline repressor induced expression of a luciferase reporter gene was readily and reproducibly achieved in both the *Xenopus* oocyte and developing embryo. This high level of expression was effectively abrogated by addition of low levels of tetracycline. The significance of this newly defined system for studies of chromatin dynamics and developmental processes is discussed.** © 2000 Academic Press

Key Words: **Xenopus; chromatin; transcription; development; gene expression; tetracycline.**

INTRODUCTION

The *Xenopus* oocyte and embryo have provided powerful model systems for the elucidation of mechanisms governing cellular and developmental processes [1–4]. In the case of the embryo, the description and use of a rapid and reliable *Xenopus* transgenic approach [5–8] have given a new perspective to *Xenopus* developmental studies [9]. This approach permits the overexpression of gene products in every cell of the organism or in a specific tissue. With this new technology comes the need to define parameters for its effective application, including the adaptation of existing transgenic methodologies. One of the invaluable features of the *Xenopus* oocyte system is its capacity to efficiently transcribe foreign genes encoded on microinjected plasmids following their assembly into chromatin [1, 10–13]. Recent evidence has suggested that processes regulating chromatin stability are linked to the transcriptional regulatory machinery (reviewed in [14]), highlighting the need to examine nuclear processes in a chromatin context. Since both transcription and replication can be assessed on templates assembled into chromatin using the *Xenopus* system, regulatory tools for use in the *Xenopus* oocyte or embryo would have important applications for the study of interactions between chromatin and the transcription and replication machinery. One such regulatory tool is the tetracycline-mediated gene expression switch.

Tetracycline-controlled gene expression was first described by Gossen and Bujard in 1992 and utilizes the very specific and high affinity binding of the *E. coli* tetracycline repressor protein (tetR) to its operator sequence (tetO) [15]. Using a fusion protein consisting of tetR fused to the VP16 activation domain (tTA) in HeLa cells, a luciferase reporter gene was activated up to five orders of magnitude and "turned off" to basal levels by the addition of low amounts of tetracycline to the tissue culture media. This tightly regulated genetic switch has been employed in a variety of studies where conditional gene expression is required. It has been used successfully in transgenic mice [16–18], where it is particularly appealing when the gene products under study are toxic or inhibitory to embryonic development. To address more diverse questions, the effective binding of tetR to its operon sequence has been exploited for purposes other than gene regulation. For example, it has been used in yeast to mark a specific region of DNA for mapping of sister chromatid separation with a tetR/GFP fusion protein bound to an array of tetO sites [19–21]. In the context of chromatin, tetR was demonstrated to form a physical boundary to nucleosome mobility in an *in vitro Drosophila* assembly system, thereby establishing a means to functionally analyze the chromatin remodeling machine CHRAC [22]. Here we describe, for the first time, the optimal conditions for successful application of this tetracycline-regulated switch for *in vivo* approaches in both the *Xenopus* oocyte and embryo.

MATERIALS AND METHODS

Antibodies and Constructs

pUHC13.3 contains seven tetO binding sites upstream of the minimal CMV promoter driving the luciferase reporter gene and pUHD15.1 expresses tTA protein. Both were generous gifts from S. Robine and have been previously described [15]. tTA mRNA was transcribed *in vitro* from the pSP65tTA plasmid constructed by in-

[1] To whom reprint requests should be addressed. Fax: 00 33 1 42 34 64 21. E-mail: almouzni@curie.fr.

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serting the tTA coding region from pUHD15.1 into pSP65 by *Eco*R1/*Bam*H1 digestion and ligation. M13E4tetO was produced by removing the five Gal4 binding sites from M13E4G5 [2] by a *Hin*dIII/*Bam*HI digest and blunt ligation to the seven tandem repeats of tetO removed from pUHC13.3 by *Xho*I/*Stu*I digestion. The sequences and orientation of constructs were confirmed by sequencing. The TetR monoclonal antibody was raised against the tetracycline-responsive transcriptional activator tTA (Clontech catalog no. 8632-1) and was used at a 1:500 dilution for Western blotting according to standard protocols.

Xenopus Microinjection Strategy

Stage VI *Xenopus* oocytes were surgically removed and treated with collagenase as previously described ([11]; for methodological reviews, see [23]). *In vitro* transcribed tTA mRNA (quantity and quality assessed by UV analysis and electrophoresis) was injected into the cytoplasm of stage VI oocytes using a Drummond Nanoject automatic injector and incubated at 16°C overnight to allow tTA protein expression and accumulation. Approximately 18 h later, the tTA-regulated luciferase reporter pUHC13.3 [15] was injected into the nucleus and oocytes were incubated +/− 200 ng/ml tetracycline hydrochloride (Sigma) for a further 5 h to allow time for chromatin assembly on the reporter plasmid and luciferase expression. Ten healthy oocytes were recovered and lysed in 100 μl of lysis buffer, and the levels of luciferase reporter activity were assessed as described in the luciferase detection kit (Perkin–Elmer). Following lysis, DNA and RNA were analyzed as previously described [2].

Fertilized *Xenopus* eggs were coinjected into one blastomere at the 2-cell stage of development with 50 pg of pUHC13.3 reporter DNA and various amounts of tTA mRNA in a total volume of 26.7–32.2 nl as previously described [3]. Embryos were incubated +/− 2 μg/ml tetracycline hydrochloride for at least 14 h at 23°C. Alternatively, tetracycline was dissolved in water and coinjected into the embryos with pUHC13.3 and tTA mRNA to give a final concentration in the embryo of approximately 500 ng/ml. Embryos were lysed at various stages of development [24] and luciferase activity assessed.

Transcription Analysis

To assess the levels of either luciferase or E4 transcript accumulated per DNA template, a reverse transcription assay was performed as previously described [2]. Accumulation of luciferase mRNA transcript from 5 ng/oocyte pUHC13.3 luciferase reporter was detected by reverse transcription from an end-labeled oligo(5′-AGCCTTATGCAGTTGCTCTC-3′) annealed to luciferase mRNA extracted from the oocyte as previously described [2]. Extension gives rise to a product of 306 nucleotides. The same method was used to detect the E4 transcript, using in this case the end-labeled oligo(5′-CTTCACACCGGCAGCCTAACAGTCAGCC-3′), which produces a major product of 100 nucleotides. Efficiency of microinjection and chromatin assembly on transcribed templates was assessed by performing a supercoiling assay in parallel on DNA extracted from the same lysate used to prepare the RNA for reverse transcription analysis. Each lane of gel represents extension product from RNA accumulated in 2 oocytes. Quantification of transcripts and DNA recovery was performed with a PhosphoImager (STORM).

Supercoiling Assay

Following lysis of oocytes, a supercoiling assay was used as a measure of the efficiency of microinjection and assembly of injected templates into chromatin as previously described [2]. Each lane of the supercoiling assay represents DNA extracted from 5 oocytes from an injection of 5 ng/oocyte. Following electrophoresis, the DNA was Southern blotted and hybridized to M13E4tetO radioactively labeled with ^{32}P by random priming (Amersham Rediprime II RPN 1633).

RESULTS

Tetracycline-Regulated Gene Expression in the Xenopus Oocyte

It has been reported that the responsiveness of tetracycline-inducible systems can vary, depending on the cell type [25, 26]. In order to determine whether conditional gene expression could be achieved in *Xenopus* using the tetracycline-regulated system, we first asked whether expression of a luciferase reporter could be induced by tTA using a transient assay. The tTA protein was produced in the oocyte following cytoplasmic microinjection of *in vitro* transcribed tTA mRNA. This approach has previously been reported to be a reliable method for introducing foreign proteins into the oocyte [27–29], where the activity of injected RNA may be further improved by flanking the RNA with untranslated regions of the *Xenopus* globin gene. Therefore, we determined the activation threshold using 5 ng of microinjected reporter DNA when coinjecting increasing amounts of tTA mRNA. First, we confirmed that tTA protein was being produced in the oocyte at levels corresponding to the increase in injected mRNA (Fig. 1A). Second, we measured accumulation of luciferase mRNA by a reverse transcription assay (Fig. 1B). When the level of luciferase transcript was assessed, it was evident that an increase in activation of transcription from this reporter could be obtained using up to 75 ng of tTA mRNA, with 5 ng of reporter DNA reaching an optimal induction between 60 and 75 ng (Fig. 1B). The amount of tTA mRNA required to reach this optimal level is within the previously reported limit of 100 ng of mRNA that can be expressed by a single oocyte [30]. Factors available for luciferase protein expression also appear to be limiting since luciferase activity reaches a peak at 45 ng of injected tTA RNA (Fig. 1C), indicating that when high quantities of DNA are required, transcription efficiency should be assessed by analysis of the resulting transcript rather than the protein product.

Luciferase activity from the pUHC13.3 reporter was subsequently used as a measure of tTA-regulated expression of low levels of reporter since it has been well characterized for transient assays in tissue culture cells [15, 31, 32]. Figure 2A illustrates the experimental strategy taken for introduction of the tTA protein and luciferase reporter and analysis of products in the *Xenopus* oocyte. With this strategy, a range of amounts of both reporter plasmid and tTA mRNA gave high levels of reporter induction (Fig. 2B), achieving almost 50-fold induction of luciferase activity above basal levels expressed from the reporter without the tTA activator. An optimal induction was reached when 250 pg of reporter and 15–22 ng of tTA mRNA were microinjected since no significant increase in activation was

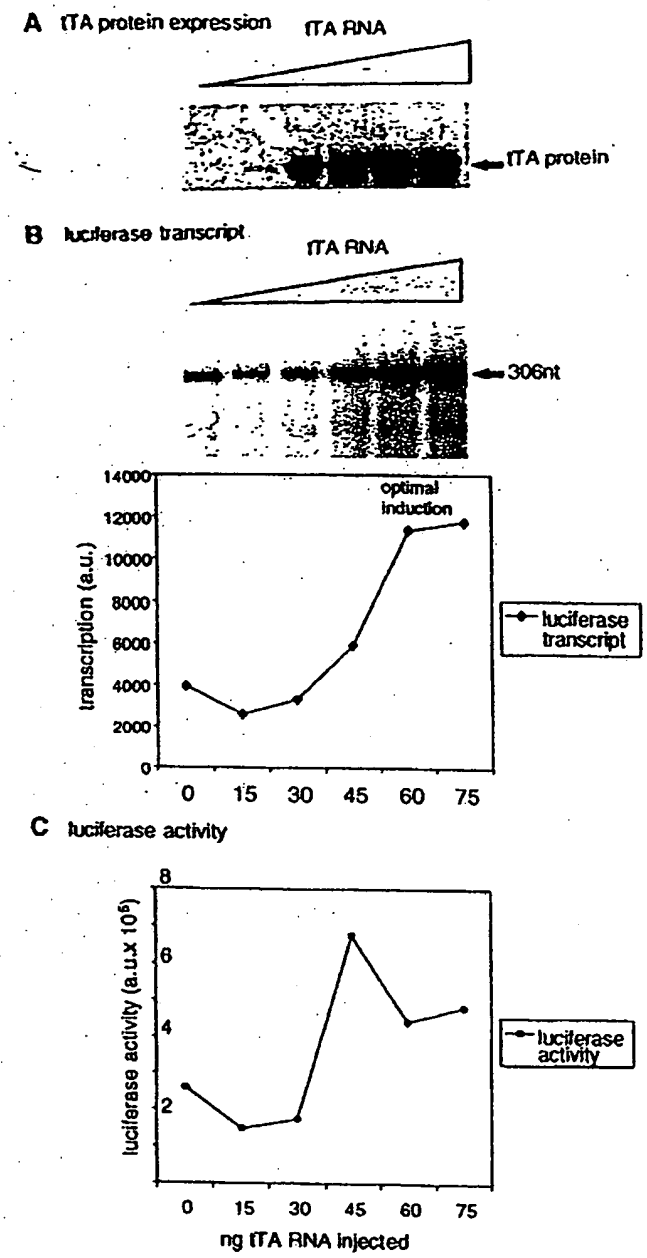

FIG. 1. Optimizing conditions for microinjection of tTA mRNA in the *Xenopus* oocyte. (A) Western analysis of *in vivo* translated tTA protein from oocyte extracts. Stage VI *Xenopus* oocytes were injected into the cytoplasm with the following increasing amounts of *in vitro* transcribed tTA mRNA: 0, 15, 30, 45, 60, and 75 ng/oocyte. Following injection, oocytes were incubated overnight for tTA protein expression and accumulation. Fifty oocytes were homogenized for each variable and centrifuged at 35,000 rpm and the clear protein extract layer was removed. Extract from the equivalent of 2 oocytes was then analyzed by Western blotting and revealed using a TetR monoclonal antibody (Clontech catalog no. 8632-1) at a 1:500 dilution and chemiluminescence according to manufacturer's instructions (Pierce SuperSignal, catalog no. 34080). (B) tTA-activated transcription from the pUHC13.3 reporter. (upper panel) Transcription of luciferase mRNA from 5 ng of pUHC13.3 luciferase reporter was detected by reverse transcription as described under Materials and Methods. The expected size of the reverse transcription product is given at 306 nt. A range of tTA mRNA of 0, 15, 30, 45, 60, and 75 ng/oocyte was injected in each case. (lower panel) Graphic illustration of accumulated luciferase mRNA, as described above, analyzed by phosphoimaging of the upper panel reverse transcription reaction. Optimal induction is indicated. (C) tTA-activated luciferase activity. Expression of luciferase from 5 ng of pUHC13.3 luciferase reporter was measured by a luciferase assay as described under Materials and Methods. A range of amounts of tTA mRNA of 0, 15, 30, 45, 60, and 75 ng/oocyte was injected as indicated.

detected despite the increased tTA RNA injected. This suggests that at this level all tTA binding sites are occupied. Significantly, addition of 200 ng/ml of tetracycline to the culture media was sufficient to completely abrogate this induction (Fig. 2B). Therefore, low levels of injected reporter DNA produced conditional gene expression, illustrating the effectiveness of this regulatory system in the *Xenopus* oocyte.

Tetracycline-Regulated Gene Expression during Development

Because of the high specificity and the low toxicity of the tetR protein and the tetracycline effector [15, 16], we wanted to determine the effectiveness of this system for use in the developing *Xenopus* embryo. Using an experimental strategy depicted in Fig. 3A, we coinjected fertilized eggs with luciferase reporter and various amounts of tTA mRNA to define the optimal parameters for tTA-driven promoter activation. Levels of injected DNA were kept to a minimum (50 pg/embryo) since higher amounts of DNA injected during early development result in low survival rates [33]. The most effective level of tTA mRNA to coinject with 50 pg of reporter was assessed at two different stages of development and determined to be 15–22 ng/embryo (data not shown). With these optimal conditions, high and reproducible levels of conditional gene expression were achieved in the developing embryo (Fig. 3B) following the midblastula transition when zygotic transcription is initiated (reviewed in [34]). At stage 11, although there is background luciferase activity expressed from the reporter plasmid, indicating that the embryos have developed beyond the MBT, there is no evidence of induction of luciferase expression by tTA, suggesting that not enough time has elapsed to establish levels of tTA protein necessary for activation of transcription. By stage 12, however, there is an effective induction of luciferase expression by tTA to over 100-fold, which is maintained through early development, with the peak of activation at stage 19 producing over 200-fold stimulation above levels with the reporter alone. By stage 27, the level of activation has dropped to 74-fold, indicating that the maximal limits of the system may have been reached by this stage. Significantly, levels of injected DNA are reported to decline following gastrulation [35], which may reflect the inability of even high levels of injected

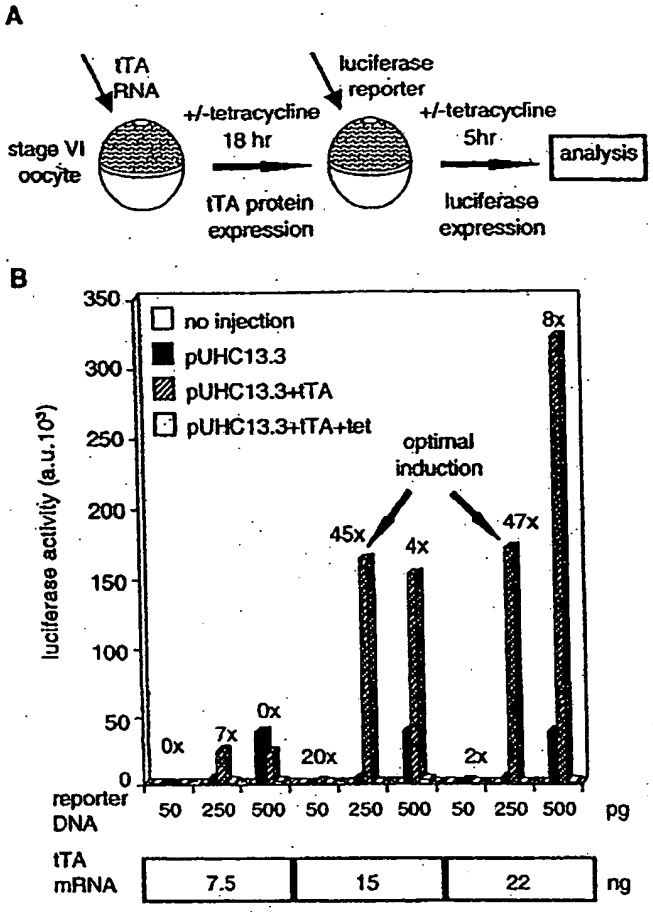

FIG. 2. Tetracycline-regulated gene expression in the *Xenopus* oocyte. (A) Experimental strategy for the tetracycline-regulated gene expression system in the *Xenopus* oocyte. Stage VI *Xenopus* oocytes were surgically removed and treated with collagenase as previously described [11, 23]. *In vitro* transcribed tTA mRNA was injected into the cytoplasm of oocytes, which were subsequently incubated at 16°C overnight +/− 200 ng/ml tetracycline hydrochloride (Sigma) to allow tTA protein expression and accumulation. Approximately 18 h later, the tTA-regulated luciferase reporter pUHC13.3 [15] was injected into the nucleus of the same oocytes and incubation continued +/− tetracycline for a further 5 h to allow time for chromatin assembly on the reporter plasmid and luciferase expression. (B) tTA induction of luciferase activity in *Xenopus* oocytes. Histograms illustrate the levels of luciferase activity, in relative light units, detected in the equivalent of 2 oocytes from the lysis of a total of 10 healthy oocytes per variable. Levels of induction and its abrogation on addition of 200 ng/ml tetracycline are shown after injecting 50, 250, or 500 pg of pUHC13.3 luciferase reporter and either 7.5, 15, or 22 ng of tTA mRNA/oocyte as indicated. Optimal induction and fold activation +tTA are indicated.

tTA mRNA to increase luciferase expression at this time. The above results indicate that the optimal induction of gene expression driven by the tTA protein using this transient assay is obtained at stage 19 of development, with high conditional activation achieved from stages 12 through to at least 27 using a ratio of 1:440 coinjected reporter DNA to tTA mRNA. We confirmed that the injected mRNA was translated into tTA protein by Western blotting of embryo extracts and that this level of mRNA was not inhibitory to early embryonic development (Fig. 3C). Injected DNA has been shown to persist for many months at low levels in the developing *Xenopus* [33, 35] probably following its integration into the host cell genome, suggesting that a mosaic pattern of induction may be achievable even at advanced stages of development using the protocol outlined in Fig. 3A. In fact, we were able to detect some activity from the luciferase reporter to the swimming larvae stage (stage 43) (data not shown).

Although it is clear that tTA can reach its tetO binding site to activate transcription in the embryo, results from these experiments indicate that tetracycline in the culture media has no effect on this activation (Fig. 3B, gray histograms). In a subsequent tetracycline titration experiment, a similar lack of effect was observed using 200 ng/ml and 0.02 mg/ml tetracycline (data not shown). Very high levels of tetracycline caused an arrest in development (at stage 13 for 0.2 mg/ml and stage 7 in the case of 2 mg/ml). Although the tetracycline-mediated shutoff of gene expression by tTA can be effectively achieved in tissue culture cells [15, 30] and in the *Xenopus* oocyte (Fig. 2B), it is not as rapidly established in whole organisms. Tetracycline can reduce levels of tTA-induced expression in transgenic mice implanted with slow-release tetracycline pellets, but this is often measured following 7 days of implantation [16] and variations in effectiveness with tissue type have been shown to exist [16–18]. We reasoned that *Xenopus* embryos could be more resistant than oocytes to the diffusion of tetracycline from the culture media. To address this question, we coinjected tetracycline with tTA mRNA and reporter DNA rather than adding tetracycline to the culture media. With this protocol, tTA-induced activation of luciferase was abolished by tetracycline even though injection of tetracycline with the reporter alone did not change the basal levels of luciferase expression (Fig. 4). The elimination of luciferase activity by tetracycline injection (compare pUHC13.3 + tTA histogram to pUHC13.3 + tTA + tet histogram) is remarkable considering the lack of tetracycline-mediated shutoff detected in Fig. 3B at a similar stage of development (stage 12, gray histogram). The tetracycline-regulated release of tetR from its recognition site can thereby be achieved using this coinjection strategy; however, optimization of this parameter will be needed to fully exploit the tetracycline-regulated system in the embryo.

Chromatin Assembly Coupled to Second-Strand Synthesis Eliminates Background Expression

To regulate gene expression in either the oocyte or as an integrated gene in a transgenic frog, the tTA protein must be competent to access its binding site on a DNA

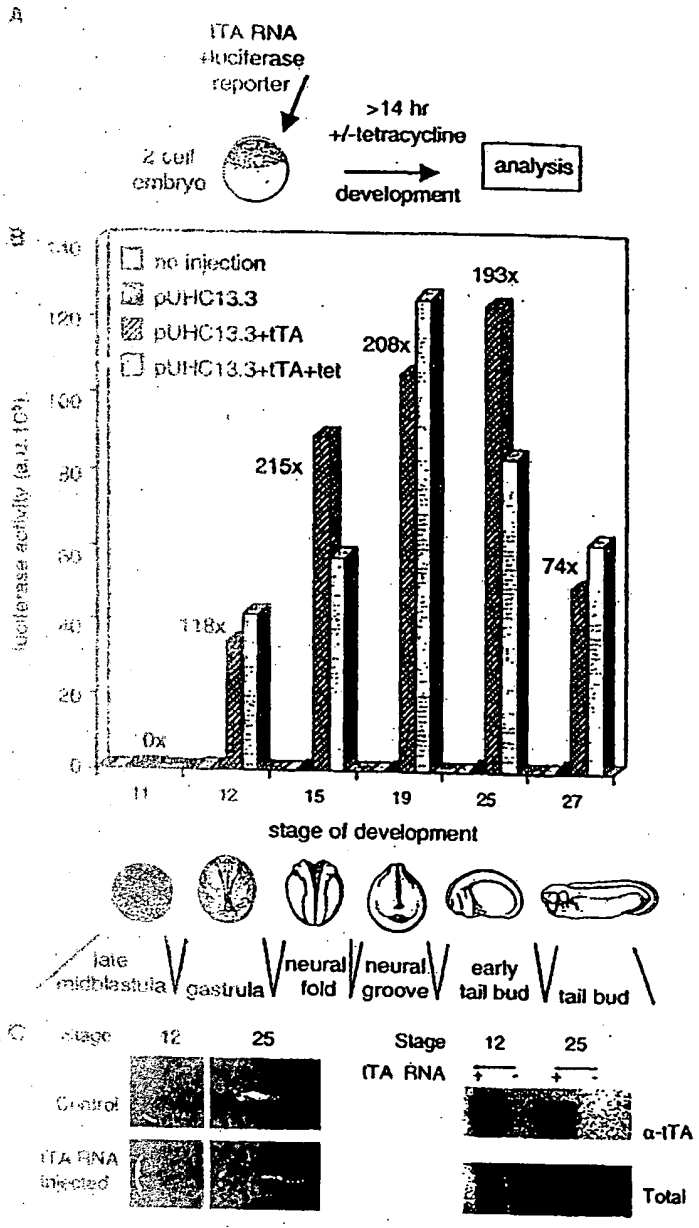

FIG. 3. Tetracycline-regulated gene expression during *Xenopus* development. (A) Experimental strategy in developing embryos. Fertilized *Xenopus* eggs were coinjected into one blastomere at the 2-cell stage of development with 50 pg of pUHC13.3 reporter DNA and various amounts of tTA mRNA in a total volume of 26.7–32.2 nl as previously described [3]. Embryos were incubated +/− tetracycline hydrochloride for at least 14 h at 23°C and lysed at various stages of development [24] to assess luciferase activity. (B) tTA induction of luciferase expression in the developing embryo. Histograms illustrate luciferase activity, in relative light units, detected from 2 embryos at various stages of development [24] as defined beneath (diagrams from Xenopus Molecular Marker Resource at http://vize222.zo.utexas.edu/). In each case, 22 ng of tTA RNA and 50 pg of pUHC13.3/embryo were coinjected and embryos incubated +/− 2 μg/ml tetracycline. Fold of activation +tTA is indicated for each developmental stage. (C) Expression of the tTA in the developing embryo. Left: pictures of control (top) and injected embryos with 25 ng of tTA RNA (bottom) were taken at the stages 12 and 25 according to [24]. Right: Western blot analysis of the corresponding embryos. The tTA protein is detected using the TetR monoclonal antibody (top panel) and the total protein is detected using Ponceau staining (bottom panel). The equivalent of 2 embryos is loaded in each lane.

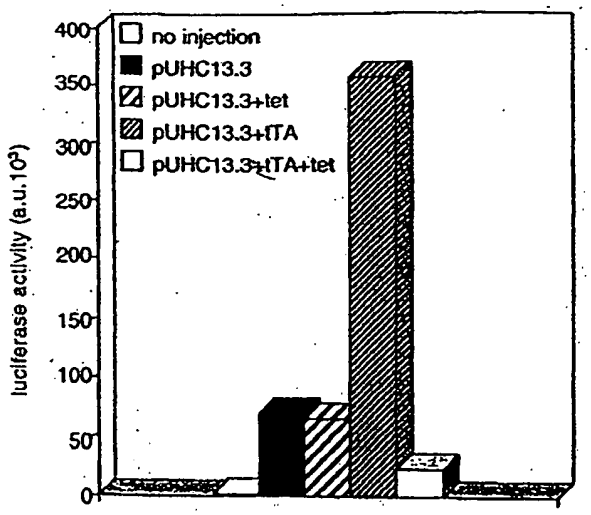

FIG. 4. Microinjection of tetracycline to regulate tTA binding. Histograms illustrate luciferase activity, in relative light units, detected from 3 embryos at stage 11–12 (midblastula) of development. In each case, 50 pg of pUHC13.3 and/or 22 ng of tTA mRNA per embryo was coinjected. Some samples, as indicated, were coinjected with a tetracycline solution to give a final concentration in the embryo of approximately 500 ng/ml.

template assembled into chromatin. Therefore, to extend the use of the induction system for analysis of microinjected reporter DNA assembled into chromatin, we further refined the conditions. Higher amounts of reporter DNA are required in this case since 1–5 ng of injected double-stranded plasmid DNA is the minimum amount necessary for effective chromatin assembly in the *Xenopus* oocyte [36]. We know from our initial experiments (Fig. 1) that 5 ng of reporter DNA is optimally activated by injection of 60–75 ng of tTA mRNA, indicating that tTA-regulated gene expression occurs on chromatinized templates, illustrating its potential as a tool either for regulation of integrated genes in transgenics or for *in vivo* chromatin studies. However, when we increase the amount of reporter DNA, background expression of luciferase is detected concomitant with a decrease in the activation potential (see Fig. 2B, 500-pg level, and Fig. 1B, basal transcription level). The high level of basal transcription from the minimal CMV promoter in this reporter construct is not surprising since genes driven by the CMV promoter are known to be highly expressed in the oocyte [2]. To eliminate this background expression from the reporter and to assess the effectiveness of this system in an integrated gene, we initiated a single-stranded (ss) template strategy. It has been shown that ss tem-

plates injected into oocytes are assembled into chromatin during the process of second-strand synthesis, which is repressive to basal transcription (initially reported in [2] and subsequently applied in [12, 29, 37, 38]). We reasoned that the introduction of the tTA-driven promoter on a ss template would provide a much more tightly regulated on/off system by repressing the effects of basal transcription. In addition, the ss template, once assembled into chromatin in the oocyte, would produce a template with the characteristics of an integrated promoter. Therefore, we constructed an M13 derivative (M13E4tetO) by removing the seven tetO binding sites from pUHC13.3 and inserting them into M13E4G5 [2] in place of the five Gal4 binding sites. Using this construct, we can compare both basal and tTA-activated transcription of the E4 gene during second-strand synthesis coupled chromatin assembly using a reverse transcription assay (Fig. 5A). When the ss template is injected, basal transcription is not detected (lane 1) whereas the double-stranded (ds) template shows background expression (lane 2) as expected. Although both the ss and ds templates were assembled into chromatin (as indicated by supercoiling, Fig. 5B), the tTA protein was able to overcome this chromatin-repressed state and activate transcription (lanes 3 and 7 for ss template and lanes 4 and 8 for ds template). Addition of 200 ng/ml of tetracycline to the oocyte culture media turned off the expression of the E4 gene (lanes 5 and 9 for ss template and lanes 6 and 10 for ds template). Therefore this ss template strategy provides a tightly regulated system to examine questions related to transcription from chromatin templates in the oocyte. It also indicates that the tetO binding sites would be accessible to tTA protein in an integrated promoter and that basal expression would be eliminated, providing an attractive mechanism for regulating overexpression of otherwise deleterious gene products in a transgenic *Xenopus*.

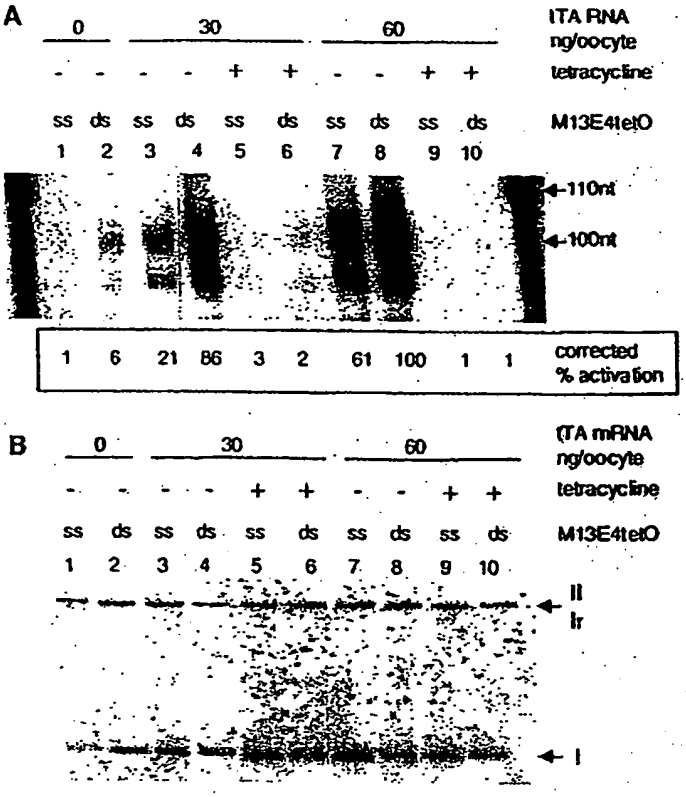

FIG. 5. Single-stranded strategy for tetracycline-regulated transcription. (A) Tetracycline-regulated E4 transcription. Transcription of the E4 gene from microinjected ss or ds M13E4tetO in *Xenopus* oocytes was detected using the reverse transcription assay described under Materials and Methods from oocyte extracts using the strategy illustrated in Fig. 2A. Either 5 ng/oocyte M13E4tetO alone (lanes 1 and 2) or in combination with 30 ng of tTA mRNA (lanes 3–6) or 60 ng of tTA mRNA (lanes 7–10) were microinjected into oocytes. Tetracycline (200 ng/ml) was added to the culture media in some cases (lanes 5, 6, 9, and 10). For each variable, 15 oocytes were lysed and the equivalent of 10 oocytes used for RNA extraction (A) and 5 oocytes for DNA extraction and supercoiling assay (B). Each lane represents mRNA extracted from 2 oocytes. The percentage activation is given for each lane corrected for amount of supercoiled template as shown in B. Marker at 110 nt is shown on right and left of gel. (B) Chromatin assembly on microinjected M13E4tetO templates. The supercoiling assay was used as a measure of chromatin assembly in the oocyte. Fifteen oocytes from each variable were pooled and lysed, with the equivalent of 10 oocytes used for RNA extraction as described above and 5 oocytes for DNA extraction and supercoiling assay. Lanes are the same as described for A. In each case, the efficiency of the microinjection was determined based on the amount of circular supercoiled DNA (I). The different forms of DNA are indicated as circular supercoiled (I), relaxed (Ir), and nicked (II).

DISCUSSION

In this report we define the optimal conditions for tetracycline-regulated gene expression in the *Xenopus* system. Information presented in Fig. 1A clearly shows that the tTA activator can be expressed to high levels in the oocyte. Importantly, the ability to assess the introduction of the tTA protein at the single-cell level in the *Xenopus* oocyte enabled us to determine that high levels of tTA (expressed from 75 ng of mRNA; Fig. 1A) can be introduced into an oocyte with no detectable deleterious effects on transcription (Fig. 1B). High levels of tTA protein can also be expressed in *Xenopus* embryos without gross developmental abnormalities (Fig. 3C). This is of interest since the tTA protein has been suggested to have toxic effects [17]. We also know that this tTA protein binds to the tetO sites since it activates luciferase expression 50-fold in the oocyte and over 200-fold in the developing embryo. The advantage of the tetR system over other activating systems such as hormone/receptor-driven systems [12, 29, 39] is the capacity to turn off activation, permitting gene activation or repression over a defined window, making it ideal for developmental applications and transgenics.

Refinements in restriction-mediated transgenic sys-

tems for *Xenopus* [5] allow overexpression of a particular gene product such as a kinase-deficient dominant-negative FGF receptor [6]. Application of tetracycline-regulated expression would improve its regulatory potential. During early stages of development, gene expression could be controlled by tetracycline injection. At later stages, when tadpoles or adults are feeding, tetracycline could be added to the food or water supply. In tetracycline-regulated mouse transgenics, a reverse rtTA induction system [18], where addition of tetracycline results in induction rather than shutoff, has overcome problems associated with the tTA system, where the half-life and clearance of the inducer are required for regulation (reviewed in [40]). This reverse system may prove to be a more effective alternative in the *Xenopus* embryo to maintain a prolonged shutoff of gene expression followed by a rapid burst of activation during a specific developmental stage or in a specific tissue. This burst of activation could be readily monitored by coupling the expression of the gene of interest to a fluorescent marker protein such as GFP.

Fluorescent markers have been used to track cellular processes by linking them to proteins and following their path during cell division or development. In such an approach, the LacI repressor fused to GFP was used to follow the localization of lactose operon operator sequences inserted into replication origin regions in *B. subtilis* [41]. An elegant series of experiments in yeast [19–21] make use of the tetracycline operator/repressor system to follow sister chromatid separation to identify factors involved in sister chromatid cohesion. The efficiency of expression and binding of tetR to its operon in *Xenopus* demonstrated in this report would facilitate the rapid application of such approaches to the study of nuclear dynamics in this higher eukaryotic system.

We are presently applying the tetracycline-regulated system to explore questions related to chromatin dynamics. Recent reports examining the action of the chromatin-remodeling machines in modifying chromatin suggest a tracking or sliding mechanism rather than the previous notion of nucleosome displacement [22, 42, 43]. In this context the binding of tetR has been used as a physical boundary to the mobility of nucleosomes during *in vitro Drosophila* chromatin assembly [22], lending further credibility to a sliding mechanism of action for CHRAC. We are using tetR as a barrier to chromatin assembly to plot the progress of bidirectional repair coupled chromatin assembly [44, 45] along DNA.

In conclusion, we have extended the application of tetracycline-regulated gene expression by defining the optimal parameters for its use in both the *Xenopus* oocyte and developing embryo. In both cases conditional expression of a gene of interest can be reliably and reproducibly achieved, adding to the already well-established usefulness of the *Xenopus* model system.

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